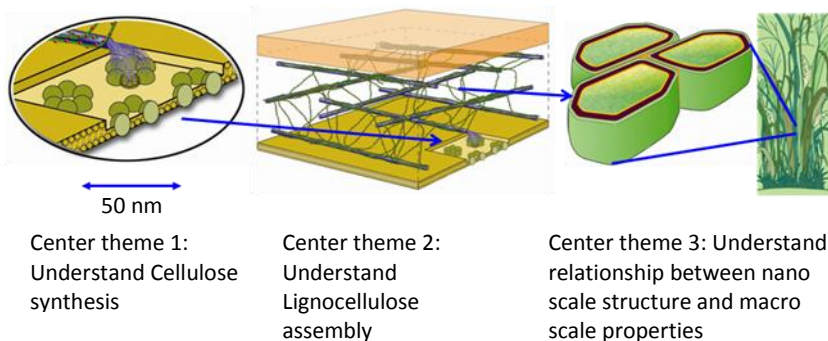**Center for Lignocellulose Structure and Formation (CLSF)**
EFRC Director: (Daniel Cosgrove)
Lead Institution: (Penn State University)

Mission Statement: CLSF will develop a detailed understanding of the nano-scale structure of lignocellulose and the physico-chemical principles of its formation. Lignocellulose is the major structural material of plant bodies and constitutes the enormously important biorenewable resource used to make building materials, paper, textiles and many polymer derivatives. It is also largest available source of biomass on Earth with the potential for conversion to transportation fuels to replace petroleum. Despite its economic significance, many basic questions about its structure and formation are unanswered. This is the focus of the center. CLSF has 3 interrelated themes, illustrated at right.

50 nm

Center theme 1: Understand Cellulose synthesis

Center theme 2: Understand Lignocellulose assembly

Center theme 3: Understand relationship between nano scale structure and macro scale properties

Theme 1 focuses on CSC, the Cellulose Synthase Complex, and the physical process of cellulose microfibril formation in plant and microbial systems. Specific objectives include:

CSC structure: Crystallize the catalytic core of Acs and Ces cellulose synthesis protein systems and develop a structural model; Analyze plant CSC from genetically engineered and/or mutant Arabidopsis lines; Work with modelers to incorporate all proven aspects of CSC structure and operation into their emerging models; Apply freeze fracture TEM to visualize the CSC and, possibly, sites of microfibril extrusion in protoplasts; and parallel work above for the bacterial CSC.

Nanoengineering: Reconstitute an active CS, using both Acs and plant CesA enzymes, into artificial membranes assembled within nanotube and nanomembrane arrays and demonstrate and manipulate CesA/CSC biochemical and biophysical function in a nano-engineered system. Use this nano-engineered platform to facilitate biophysical spectroscopic studies by providing macroscopic alignment for improved resolution, long-term sample stability, and feasibility of examination of the same sample by NMR, EPR, IR, fluorescence, etc. Refine structural models by combining experimental spectroscopic data with structural predictions of the computational modeling, and, in turn, refine the developed models.

Computational modeling: Predict secondary and three-dimensional structure of an individual CesA protein. Build a prototype computer model of CesA packing within the CSC. Explore the packing of predicted transmembrane helices in a membrane using multiscale molecular dynamics modeling. Predict the structure of the "rosette" using molecular mechanics simulations. Model the structure of crystalline cellulose and how crystallization occurs.

Theme 2 focuses on the structure and assembly of lignocellulose from its constituent components (cellulose, hemicellulose, lignin). Objectives include:

Binding and assembly studies: Characterize the dynamics and energetics of specific cellulose-polysaccharide-protein-enzyme-lignin binding interactions using isothermal titration calorimetry (ITC) and surface plasmon resonance techniques. Explore the dependence of binding parameters on the form of cellulose and the details of xyloglucan, arabinoxylan and lignin structure. Proteins and enzymes include expansins, and related nonenzymatic proteins that alter cell wall rheology, and cellulases. Combine data with molecular modeling results to understand the key molecular elements of cellulose-matrix binding

interactions. Correlate self-assembly and binding with structure enabling further correlations with results from ITC, vibrational spectroscopy, and computational modeling.

Model systems for 3D assembly studies: Develop model three dimensional synthetic plant cell wall systems implementing aligned cellulose fibrils in a flow cell. Introduce compounds such as hemicelluloses, lignins, pectins, etc., into the chamber and assess the impact on assembly. Develop a plant protoplast model system for studying the initial stages of cellulose synthesis, cellulose structure, and cell wall polymerization in Arabidopsis and *Populus* cells. Control the crystallinity of cellulose through genetic modification to significantly improve the enzymatic digestibility of cellulose. Identify novel cellulose structure in *Acetobacter xylinum* mutants through genetic modification and culture conditions. Perform computational modeling of interactions and assembly.

Spectroscopy and scanning probe microscopy studies: Elucidate cellulose-carbohydrate interactions with experimental and computational IR using models generated by the center to address such issues as whether or not the carbohydrate binds crystalline or amorphous domains. Experimentally study the effect of lignin type (degree of branching, molecule size, etc.) and reactant sequence on the solubility of xylan in cellulose-xylan-lignin complexes. Perform batch syntheses of collections of biomimetic cell wall complexes that can be recovered for further evaluation of how chemistry and microstructure relate to macroscopic properties. Explore the use of atomic force acoustic microscopy for analyzing cell wall mechanical properties and their dependence on cell wall structure and the modification by expansins, xylanases and other cell wall-loosening enzymes.

Theme 3 focuses on the development and validation of a multiscale model that will bridge the basic nano and molecular scale knowledge gathered in themes 1 and 2 to real-world applications including drying and chemical/enzymatic degradation. The following are specific objectives:

Nanoscale characterization and modeling of cell wall structure: Quantify cell wall composition (cellulose, hemicellulose, lignin, and pectin) of model and natural plant materials in order to define components for multiscale modeling. Characterize macroscale thermal and mass transport properties of natural and model plant materials. Perform computational multiscale modeling with application to structural, mechanical, thermal and transport properties/processes. Characterize biomass degradation by enzymatic or chemical means under controlled conditions. Apply multiscale models to interpret the degradation processes as impacted by nanoscale lignocellulose structure; Extend atomistic modeling to larger length and time scales by use of a coarse-grained model which incorporates atomistic detail; Develop a coarse grained simulation model for cellulose structure and crystallization. Identify 4-5 model structures for study using small angle neutron scattering. Correlate cellulose crystallinity and fibril structure with material properties. Characterize interdiffusion of water and deuterated polysaccharides in interfacial regions using neutron reflectivity.

Center for Lignocellulose Structure and Formation	
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